UNITED STATES PATENT AND TRADEMARK OFFICE

Examiner: VIVLEMORE, Tracy Ann Art Unit: 1635

Re:	Application of:	ZHAO, Hong, et al.
	Serial No.:	10/822,205
	Filed:	April 9, 2004
	For:	**POLYMERIC OLIGONUCLEOTIDE PRODRUGS**
	Confirmation No.:	3686

DECLARATION UNDER 37 CFR 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria VA 22313-1450

Sir:

I, Hong Zhao, declare and state:

1. My educational background includes a Ph.D. in Organic Chemistry from Rutgers-the State University of New Jersey in 1997; an M.S. Degree in Organic Chemistry from Rutgers-the State University of New Jersey in 1994; and a B.S. Degree in Radio Chemistry from Fudan University in Shanghai, China in 1988.

2. I am presently Director of Organic & Medicinal Chemistry at Enzon Pharmaceuticals, Inc. in Bridgewater, NJ, the assignee of the above-identified patent application, U.S. Ser. No. 10/822,205.

3. My expert opinion and conclusions as set forth in this Declaration are based upon my familiarity with the invention taught by the above-identified patent application, for which I am a co-inventor, together with my expertise and experience in the field of polymeric prodrug technology as evidenced by more than 30 publications and 21 years of research in academia and industry relating to organic and medicinal chemistry and prodrug technology.

4. My further professional experience and publications are summarized in my Curriculum Vitae, which is attached as Exhibit 1.

5. I have read and understood the Examiner's Office Action of October 1, 2008, which rejects all of the pending claims. In particular, I understand that the Examiner has taken the position that the claimed polymeric prodrugs for nucleic acids delivery is obvious over prior art. In this regard, the Examiner relied on three references: (1) Teng et al. (US Patent No. 6,887,906); (2) Greenwald et al. (US Patent No. 6,303,569); and (3) Dandliker et al. (US Patent No. 5,707,813). I have also read these references and understand their respective disclosures.

6. I believe that I am well-qualified as an expert in the field to analyze these references and to render an opinion why the polymeric prodrugs claimed in the present application are not obvious over the combination of the references relied upon by the Examiner to reject the examined claims.

7. The first purpose of this Declaration is to provide the Examiner with an analysis as to the references relied upon by the Examiner to reject the claims and an opinion as to why one having ordinary skill in the art would not have been motivated to prepare the claimed invention according to the teachings of the references.

8. The second purpose of this Declaration is to provide the Examiner with unexpectedly superior results in the claimed oligonucleotide delivery system compared to prior art.

Opinion Regarding References cited in Office Action

9. I have studied the Examiner's Office Action, which rejects all of the Applicants' pending claims. I understand that the Examiner has rejected the claims for being obvious over the descriptions found in Teng et al. (U.S. Patent No. 6,887,906), Greenwald et al. (U.S. Patent

No. 6,303,569) and Dandliker et al. (U.S. Patent No. 5,707,813). In particular, the Examiner has concluded that it would have been obvious to one of ordinary skill in the art at the time of the invention to produce the bcl-2 sequence of the Teng patent in a polymeric prodrug, as taught by the Greenwald patent, and use hexylamine linkers as a component of the prodrug. In this regard, the Examiner indicated in the paragraph bridging pages 6-7 of the Office Action that the Greenwald patent taught all of the elements of the claimed invention including the L_2 group of the formula of the claims defined as a C2-C10-containing bifunctional spacer. My review of the three patents leads me to the conclusion that the Examiner's proposal does not correspond to the teachings included in the patents.

10. In my opinion, one skilled in the art would not have been motivated to combine the teachings of the references relied upon by the Examiner to produce the claimed polymeric oligonucleotide delivery system. My opinion is based on my extensive experience as a researcher in the field of drug delivery technology, including various prodrugs. The reasons for my opinion, including analysis of the references, are set forth in the following paragraphs.

11. I have carefully reviewed the Teng patent. The Teng patent teaches a non-covalently linked delivery system for the delivery of oligonucleotides. The delivery system includes a non-covalently linked lipid carrier. The delivery system is such that oligonucleotides admixed with lipid carriers such as fatty acids and bile salts are transported to the target area in the body. The lipid carriers help oligonucleotides cross the cell membrane and, to some extent, protect from degradation. Various lipids-based delivery formulations are described in the Teng patent in detail in Example 3, "Preparation of formulations comprising oligonucleotides and fatty acids"; Example 5, "Preparation of formulations comprising oligonucleotides and bile salts"; and Examples 7 and 16. Oligonucleotides admixed with the lipid carriers are released from the carriers in the body. The release rate of oligonucleotides from the carriers in the Teng patent is not sufficiently controlled, but rather dependent on the kinds and ratios of lipid carriers.

12. I have also reviewed the Greenwald patent. The oligonucleotide delivery system taught in the Greenwald patent is different from that in the Teng patent. The Greenwald patent teaches a polymeric prodrug technology for the delivery of various biologically active agents. The delivery system is based on a polymeric double prodrug. A target drug is covalently linked to a polymeric delivery system via a releasable linker. The polymeric system solubilizes any insoluble target drug and delivers the target drug into the body. The target drug is hydrolyzed and released from the polymeric delivery system at a sufficiently controlled rate. The release rate and bioavailability of the target drug is attributed to the releasable linker employed in the polymeric delivery system. The polymeric prodrug system used in the Greenwald patent is not considered by one of ordinary skill in the art to be comparable to the lipid-based non-covalent carrier system in the Teng patent.

13. I have also carefully reviewed the Dandliker patent. The Dandliker patent relates to an oligonucleotide probe for *in vitro* application. The oligonucleotide probe of the Dandliker patent is a modified oligonucleotide in which an oligonucleotide is permanently linked to a fluorophore which has a macrocyclic multidendate ligand structure. The modified oligonucleotide of Dandliker is for *in vitro* application such as sandwich hybridization. The modified oligonucleotide of the Dandliker patent is such that two polyethylene glycol ligands are attached to the central atom of the fluorophore, and a hexyl amine-modified oligonucleotide alternative to a native form can be attached to the fluorophore. See Example 2 and Figure 2. It should be noted that the oligonucleotide of the Dandliker patent is attached to the fluorophore permanently as desired as a reliable marker for *in vitro* assay. The oligonucleotide of the Dandliker patent is not released from the fluorophore.

14. It is my understanding that the Examiner has concluded that the Teng patent taught oligonucleotides in prodrug form, and that one skilled in the art would have been motivated to produce an oligonucleotide in a polymeric prodrug form according to the teachings of Teng and Greenwald.

15. Contrary to the Examiner's proposal, it is my belief as an expert in the field that one skilled in the art would not have been motivated to combine the teachings of Teng and Greenwald to produce the alleged polymeric prodrug because a polymeric prodrug form of oligonucleotides is considered undesirable to Teng's lipids-based non-covalent oligonucleotide delivery system. It is not the case that one skilled in the art could have combined the elements as claimed by known methods with no change in their respective functions. In particular, the Teng patent described that an oligonucleotide in prodrug form as an alternative to that in native form can be employed in the Teng patent's lipids-based delivery system. At column 17, line 17 through column 20, line 1, Teng described various oligonucleotide alternatives including the alleged prodrug in the section entitled

5. Bioequivalents

A. Pharmaceutically Acceptable Salts;
B. Oligonucleotide Prodrugs;
C. Olignucleotide Deletion Derivatives;
D. Ribozymes;
E. Other Oligonucleotide Compounds.

In Teng's delivery system, oligonucleotides are mixed with sufficient amounts of lipid carriers. The bulky size of the proposed polymeric oligonucleotide prodrug is disadvantageous to formulate complexes of lipids-based carriers and polymeric oligonucleotides. One skilled in the art would consider that use of the polymeric prodrug in Teng's delivery system would negate any advantage pertaining to Teng's lipid-based delivery system or vice versa. Accordingly, the Teng patent described an example of an oligonucleotide in a non-polymeric prodrug form, SATE-modified oligonucleotide. In addition, polyethylene glycol in the polymeric prodrug of Greenwald renders the prodrug hydrophilic. This hydrophilic feature impedes formulation of complexes between hydrophobic lipids-based carriers and the hydrophilic polymeric oligonucleotide. The Teng and Greenwald patents use considerably incompatible approaches to the delivery of nucleic acids. I believe that one skilled in the art would consider that Teng's approach would cancel any benefit of the Greenwald patent or *vice versa*. Moreover, the suggested combination of references would require a substantial change in the operating principle of Teng's delivery system. Thus, it is my opinion as an expert in the field that it is unlikely for one skilled in the art to modify oligonucleotides of Teng, as taught by Greenwald, to

produce polymeric oligonucleotide prodrugs. For instance, one would not modify nucleic acids in a lipid carrier with a hydrophilic polymer conjugation.

16. It is important to note that the Examiner's position pertaining to the Greenwald patent is inaccurate with respect to the L_2 group of the Greenwald patent. In particular, the Examiner's remarks pertaining to the Greenwald patent are found in the paragraph bridging pages 6-7 of the Office Action as follows:

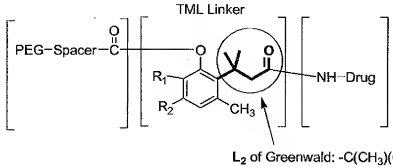
> These arguments are unpersuasive because contrary to applicants' assertions, Greenwald et al. do teach the claimed configuration of elements. As stated in the rejection, Greenwald et al. teach at columns 2-3 a prodrug comprising a polymer region designated as R_{11}, a linker comprising an aromatic group (which is equivalent to L_1 of the instant claims), a spacer designated as L_2 (which is equivalent to L_2 of the instant claims) and a drug component designated as B (which can be an oligonucleotide). Greenwald et al. further define L_2 at column 3 as spacers containing more than 2 carbons. Since Greenwald et al. explicitly teach polymeric prodrugs with the components found in the claims and further define L_2 to be a spacer group that comprises 2-10 carbons, those in the art are not left to try to prepare indeterminate linkage combinations of polymeric prodrugs and non-polymeric prodrugs, all that is needed to produce the claimed compounds is found in the cited references and could be combined with reasonable expectation of success to form the claimed compounds. (Emphasis added).

I have emphasized the portion relevant to my explanation of Greenwald. The aforementioned linker and spacer of Greenwald patent are not equivalent to the L_1 and L_2 groups of the formula in the claims of the present application, as proposed by the Examiner. The combined moiety of the aforementioned linker and spacer of Greenwald corresponds to the claimed L_1 group.

17. I will now explain the polymeric prodrug of the Greenwald patent as it pertains to the claimed invention. The Greenwald patent provided a polymeric double prodrug including a releasable linker based on a trimethyl lock lactonization which is known in the art as "TML". The spacer designated as L_2 in the Greenwald patent is part of the releasable TML linker corresponding to part of the L_1 group of the present application. The L_2 group in the Greenwald patent is not equivalent to the L_2 group of the present application, the C2-C10-containing spacer.

18. The polymeric double prodrug of the Greenwald patent releases a drug by an esterase cleavage, followed by a laconization-based cleavage. The lactonization-based cleavage in the releasable TML linker occurs when a nucleophile oxygen (O) is about three to six atoms apart from the electrophile carbonyl group (C(=O)), and the nucleophile and electrophile react to form a cyclized lactone. A schematic release mechanism of the TML double prodrug is described in the section entitled "4. Lactonization and Native Drug Regeneration" found at column 8, line 31 through column 9, line 14 of the Greenwald patent. The Greenwald patent, at column 5, lines 1-8 and 35, defined the L_2 group required for the lactonization-based cleavage as $-C(R_1)(R_4)-[CH_2]_p-G-$, wherein G is $C(=Y_1)$ and (p) is zero, one or two. Accordingly, the L_2 of Greenwald patent has a structure of $-C(R_1)(R_4)-C(=Y_1)-$, $-C(R_1)(R_4)-CH_2-C(=Y_1)-$, or $-C(R_1)(R_4)-[CH_2]_2-C(=Y_1)-$.

19. The co-inventors of the Greenwald patent authored an article (J. Med. Chem. 2000, 43:475-487) describing the release mechanism of the TML double prodrugs. The article is attached hereto as Exhibit **2**. A simplified structure of the TML double prodrug found in Figure 1 on page 475 of the article is reproduced as follows:



The aforementioned portion of the TML linker corresponding to the L_2 of Greenwald patent is emphasized in bold. A similar schematic release mechanism of the TML double prodrug is also described in the section entitled "4. Lactonization and Native Drug Regeneration" found at column 8, line 31 through column 9, line 14 of the Greenwald patent. As shown above, the L_2 group of the Greenwald patent is part of the TML linker, not the L_2 group of the claimed invention, the C2-C10-containing spacer.

20. The Dandliker patent taught permanent linkage of the hexyl amine-modified oligonculeotide to a fluorophore which is attached to PEG ligands. The teachings of the Dandliker patent are squarely incomparable to those of Greenwald in which a target drug should be released from the polymeric prodrug. The Teng, Greenwald, and Dandliker patents taught incompatible systems which would negate any advantage pertaining to each patent.

21. It is my conclusion that Teng, Greenwald, and Dandliker, whether taken alone or in any combination, described neither the claimed C2-C10-containing L_2 spacer in a polymeric prodrug, nor the conjugation of the C2-C10-containing L_2 spacer-modified oligonucleotide to a polymer via a releasable linker as required in the claimed polymeric prodrugs.

Unexpectedly Superior Oliognucleotide Delivery System

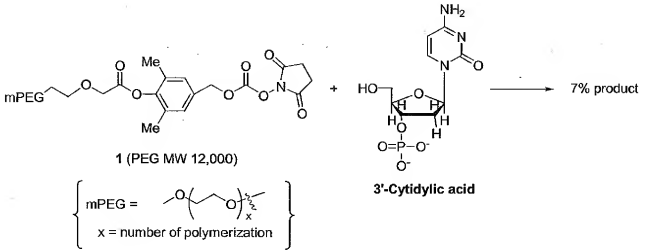
22. It is important to appreciate the unexpected and superior properties of the claimed polymeric system for the delivery of oligonucleotides. I believe that those advantageous properties are as a result of including the L_1 and L_2 linkage now claimed. The claimed polymeric prodrug eliminates needs for transfection agents which are commonly required for the delivery of sufficient amounts of oligonucleotides into the cells according to prior art. This feature is advantageous in therapy employing oligonucleotides since toxicity associated with transfection agents is one of the major hurdles deterring development of therapy associated with oligonucleotides. It is very important for such polymeric oligonucleotide delivery systems to be able to load sufficient amounts of oligonucleotides in great yield and purity by conjugating oligonucleotides to an activated polymer.

23. In this regard, I have first attempted to produce a polymeric prodrug in which there are three elements: (1) a polymer, (2) a releasable linker and (3) an oligonucleotide, without the claimed L_1 and L_2 linkage. The attempted oligonucleotide delivery system corresponds to that of the prior art. In particular, I have attempted to conjugate an oligonucleotide directly to a polymer containing a releasable linker based on a benzyl

elimination known as "RNL" in the art. The RNL linker is a similar releasable linker system which compares favorably to the TML linker disclosed in the Greenwald patent. The RNL linker releases the conjugated nucleic acid(s) in a manner similar to the TML linker in the Greenwald patent. The RNL linker corresponds to a suitable L_1 comparative example of my invention. Additional details of the RNL linker and release mechanism thereof are described in U.S. Patent No. 6,180,095 (Exhibit **3**), and an article authored by the inventors of the present application (Greenwald et al. J. Medicinal Chemistry, 1999, 42:3657-3667). The article is attached hereto as Exhibit **4.**

24. Direct conjugation of a nucleic acid to an activated polymer containing the RNL linker provided a polymeric conjugate in *only about 7% yield.* In the conjugation, 3'-cytidylic acid (10 mg, 30.9 μmol) was dissolved in 10 mL of PBS buffer (pH 6.5) and an activated PEG (compound **1**, 380 mg, 30.9 μmol) was added to the solution. The reaction mixture was stirred overnight at room temperature. The reaction was monitored and analyzed by HPLC at 280 nm and the amount of PEG conjugation was calculated based on the area under the curve for nucleotide and product. After about 18 hours stirring at room temperature, only about 7% of cytidylic acid conjugated to PEG. The reaction is schematically shown in Reaction Scheme A.

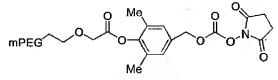
Reaction Scheme A:



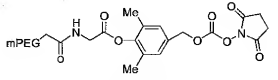
Likewise, direct conjugation of an oligonucleotide to an activated polymer containing the RNL linker alone did not successfully provide a polymeric oligonucleotide prodrug. As such, prior art

systems employing only the L_1 portion were not sufficiently successful in conjugating nucleic acids.

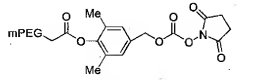
25. On the other hand, when oligonucleotides including the claimed L_2 linker were reacted with polymeric systems having the releasable L_1 linker, *more than about 30% and up to 90 %* conjugation was achieved. Examples of the activated polymers which successfully conjugated to the modified oligoncleotides are as follows:



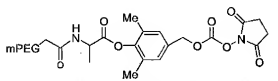
1 (PEG MW 12,000),



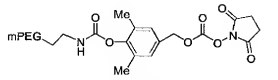
10 (PEG MW 20,000),



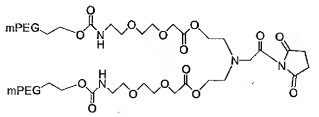
20 (PEG MW 20,000),



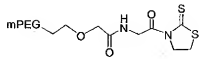
32 (PEG MW 20,000),



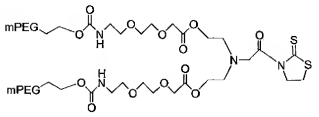
34 (PEG MW 20,000),



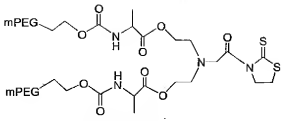
23 (PEG MW 12,000),



27 (PEG MW 20,000),



8 (PEG MW 12,000),



25 (PEG MW 12,000)

26. For example, about 40% of an oligonucleotide modified with L_2 being a hexyl amine spacer (compound **2**) conjugated to the aforementioned polymer (compound **1**), thereby providing the polymeric oligonucleotide prodrug (compound **3**). Note that the hexyl amine spacer was attached to the oligonucleotide at the 5' phosphate position. Details of the reaction and result are described in Example 1 in the specification. Also see FIG. 1. In a conjugation using another kind of polymer (compound **10**, PEG 20,000 da) in place of compound **1**, about 57% of an oligonucleotide modified with the hexyl amine spacer (compound **13**) conjugated. The oligonucleotide included photorothioate linkages. Details of the reaction and result are described in Example 7 in the specification. Also see FIG. 3. Further, about 60% of another kind of oligonucleotide modified with the hexyl amine spacer (compound **15**) conjugated to the polymer (compound **10**). Note that the hexyl amine spacer was attached to the oligonucloetide at the 3' phosphate position, and the oligonucleotide included photorothioate linkages. See FIG. 4. About 67% of another kind of oligonucleotide modified with the hexyl amine spacer (compound **AS1**) conjugated to the polymer (compound **10**). The oligonucleotide included photorothioate linkages. Details of the reaction are described in Example 9. Similarly, about 67% of another kind of oligonucleotide modified with the hexyl amine (compound **AS2** or **AS3**) conjugated to the polymer (compound **10**). See FIG. 5. The oligonucleotide (compound **AS3**) included methyl-modified bases (Me-dC). Further, about 90% of the oligonucleotide (compound **13**) conjugated to another kind of polymer containing the RNL linker (compound **20**, PEG 20,000 da). See FIG. 6.

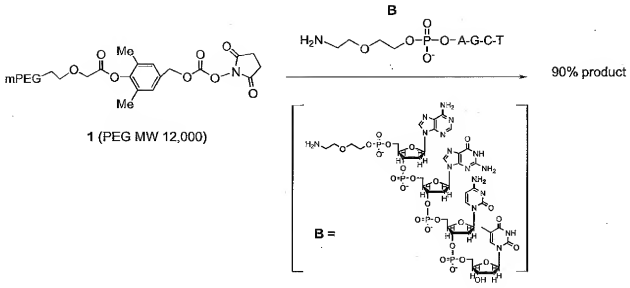
27. In addition, another kind of polymer containing a bicine-based releasable linker in place of the RNL releasable linker was used in conjugation to the oligonucleotides. About 30 %

of the oligonucleotide containing the hexyl amine linker (compound **13**) conjugated to a polymer containing the bicine-based releasable linker (compound **23**). See FIG. 7.

28. The modified oligonucleotides also successfully conjugated to a polymer including another kind of activating group such as a cyclic imide thione group instead of the N-hydroxysuccinimide group (NHS). The modified oligonucletides (compound **13**) conjugated to a cyclic imide-thione activated polymer (compound **8**) including the same bicine linker as in the aforementioned compound **23** and provided the product (compound **9**) in the yield ranging from about 30 to 50 %. See FIG. 2. About 30% of the oligonucleotide (compound **13**) conjugated to the cyclic imide-thione activated polymer including another kind of the bicine linker (compound **25**). See FIG. 7. About 65% of the modified oligonucleotide (compound **AS1**) conjugated to the cyclic imide-thione activated polymer including another kind of a releasable linker (compound 27). See FIG. 8.

29. Further, oligonucleotides attached to another kind of L_2 linker successfully conjugated to the polymers. For example, about 90% of an oligonucloetide attached to a $CH_2CH_2OCH_2CH_2NH$ spacer in place of the alkylamine spacer (e.g., $(CH_2)_6NH$) successfully conjugated to a polymer including the RNL linker. An oligonucleotide modified with a $CH_2CH_2OCH_2CH_2NH$ spacer (compound **B**, 3.42 mg, 2.5 µmole) was dissolved in 10 mL of PBS buffer (pH 6.5) and an activated PEG (compound **1, 307 mg, 25 µmol**) was added slowly over 2 hours to the solution. The reaction mixture was stirred overnight at room temperature. The reaction was monitored and analyzed by HPLC at 280 nm and the amount of PEG conjugation was calculated based on the area under the curve for oligonucleotide and product. After about 20 hours stirring at room temperature, it was observed that about 90% of oligonucleotide conjugated to the polymer. The conjugation was shown below.

Reaction Scheme B:



30. As confirmed by the experiment data, the claimed invention is an improved polymeric oligonucleotide prodrug as compared to prior art in loading higher amounts of oligonucleotides. At least four times and up to 12.8 times of the nucleic acids conjugated to the polymers, as compared to prior art systems. According to the claimed invention, nucleic acids either at 3' or 5' position, as desired, attached to the C2-C10-containing L_2 spacer conjugated to a variety of polymers including different releasable linkers and/or activating groups. I believe that the low yield of conjugated polymeric product according to prior art was attributed in part to the fact that oligonucleotides include multiple functional groups such as phosphates and OH groups. I also believe that these multiple functional groups have similar reactivity, and did not only provide a mixture of conjugates due to non-specific reaction, but also hindered the overall conjugation. On the other hand, according to the claimed invention, greater amounts of nucleic acids conjugated to various polymers, forming a uniform linkage between the releasable linker and the C2-C10-containing L_2 spacer, thereby providing a uniform product because of the claimed L_1 and L_2 linkage. This feature is advantageous to the preparation of uniform pharmaceutical formulations which are strictly regulated for reliable and safe dosing in the treatment associated with oligonucleotides.

31. None of the references taught an oligonucleotide delivery system containing the linkage of the releasable L_1 linker and the C2-C10-containing L_2 spacer between a polymer and an oligonucleotide. Moreover, the ordinary artisan would have had no reasonable expectation of success in preparing the improved polymeric prodrugs in surprisingly great yield by including the linkage of the L_1 linker and the L_2 spacer in the polymeric prodrug. The combination yielded more than predictable results to one of ordinary skill in the art. In addition, the claimed linkage of the releasable linker and the spacer is advantageously superior because the linkage allows uniform loading of the oligonucleotide per polymeric prodrug. For example, one equivalent of oligonucleotide conjugated to one equivalent of polymer through the linkage. When an alkyl amine spacer is employed, a releasable linker is selectively linked to the amine of the spacer rather than one or more multiple functional groups on oligonucleotides (e.g., amine groups on the base, hydroxyl groups on the sugar, or phosphate of the backbone as in prior art). Direct conjugation of an oligonucleotide to a polymer via a releasable linker according to prior art would produce a mixture of compounds with different conjugation linkages because the multiple functional groups on oligonucleotides would react with the releasable linker. The conjugation of prior art would also result in different loading of the oligonucleotides per prodrug. For example, one equivalent of oligonucleotide would conjugate to multiple equivalents of polymer. These features would not be helpful in dosing. Thus, the inventive polymeric prodrug including the linkage of the L_1 releasable linker and the L_2 spacer provides means for a more reliable and flexible dosing regimen to clinicians.

32. I have provided evidence that one skilled in the art could not have prepared an unexpectedly superior and improved polymeric oligonucleotide delivery system. I believe that the inventive polymeric prodrug provides superior means for the delivery of oligonucleotides into the body compared to the prior art, and is not obvious over the references relied upon by the Examiner.

33. I further declare that all statements made herein of my knowledge are true, and that all statements made on information and belief are believed to be true, and further that these statements were made with the knowledge that willful and false statements and the like so made are punishable by fine or imprisonment, or both, under ' 1001 of Title 18 of the U.S. Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

4/1/09

Date

Hong ZHAO, Ph.D.